Hysteroscopy in recurrent in-vitro fertilisation failure (TROPHY)

El-Toukhy, Tarek; Campo, Rudi; Khalaf, Yacoub; Tabanelli, Carla; Gianaroli, Luca; Gordts, Sylvie; Gordts, Stephen; Mestdagh, Greet; Mardesic, Tonko; Voboril, Jan; Marchino, Gian Luigi; Benedetto, Chiara; Al-Shawaf, Talha; Sabatini, Luca; Seed, Paul T; Gergolet, Marco; Grimbizis, Grigoris; Harb, Hoda; Coomarasamy, Aravinthan

DOI:
10.1016/S0140-6736(16)00258-0

Document Version
Peer reviewed version

Citation for published version (Harvard):
El-Toukhy, T, Campo, R, Khalaf, Y, Tabanelli, C, Gianaroli, L, Gordts, S, Gordts, S, Mestdagh, G, Mardesic, T, Voboril, J, Marchino, GL, Benedetto, C, Al-Shawaf, T, Sabatini, L, Seed, PT, Gergolet, M, Grimbizis, G, Harb, H & Coomarasamy, A 2016, 'Hysteroscopy in recurrent in-vitro fertilisation failure (TROPHY): a multicentre, randomised controlled trial', *The Lancet*, vol. 387, no. 10038, pp. 2614-2621. https://doi.org/10.1016/S0140-6736(16)00258-0

Hysteroscopy in recurrent in vitro fertilisation failure: a multi-centre randomised controlled trial [TROPHY Trial]

Authors:

Tarek El-Toukhy[1]* **MRCOG**, Rudi Campo[2], **MD,** Yacoub Khalaf[1], **FRCOG,** Carla Tabanelli[3], **MD,** Luca Gianaroli[3], **MD,** Sylvie S. Gordts[4], **MD,** Stephan Gordts[4], **MD,,** Greet Mestdagh[2], **MD,,** Tonko Mardesic[5], **MD,** Jan Voboril[5], **MD,** Gian Luigi Marchino[6], **MD,** Chiara Benedetto[6], **MD,** Talha Al-Shawaf[7], **FRCOG,** Luca Sabatini[7], **MRCOG**, Paul T Seed[8], **MSc CStat,** Marco Gergolet[9], **MD,** Grigoris Grimbizis[10], **MD,** Hoda Harb[11], **PhD**, Arri Coomarasamy[11] **FRCOG**

[1] Assisted Conception Unit, Guy's and St Thomas' Hospital NHS Foundation Trust, London, United Kingdom

[2] Ziekenhuis Oost Limburg, Assisted Conception Unit, Genk, Belgium.

[3] SISMER, Assisted Conception Unit, Bologna, Italy.

[4] Leuven Institute for Fertility and Embryology, Tiensevest, Leuven, 168 3000, Belgium.

[5]The Sanatorium Pronatal, Assisted Conception Unit, Prague, Czech Republic.

[6] Obstetrics and Gynaecology Department, University of Turin, Torino, Italy.

[7] Centre for Reproductive Medicine, Barts Health NHS Trust, London, United Kingdom.

[8] Division of Women's Health, Women's Health Academic Centre, King's College London and King's Health Partners, St Thomas' Hospital, London, United Kingdom.

[9] Reproductive Surgery Special Interest Group, European Society of Human Reproduction and Embryology, Brussels, Belgium

[10] First Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Greece

[11]Institute of Metabolism and Systems Research; Tommy's National Centre for Miscarriage Care and Research, University of Birmingham, Birmingham, United Kingdom

*Address for correspondence:

Assisted Conception Unit, 11th Floor Tower Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT, UK

E-mail: tarek.el-toukhy@gstt.nhs.uk

Tel: +442071887188 ext: 52298

Abstract

Background:

The success rate of in vitro fertilisation remains modest and many patients undergo multiple treatment cycles. Previous studies suggested in vitro fertilisation outcome could be improved in patients who have experienced recurrent implantation failure if hysteroscopy was performed before starting a treatment cycle. However, those studies were of limited quality and a definitive randomised trial was needed.

Methods:

The TROPHY trial was a single-blind multi-centre randomised controlled trial conducted in eight hospitals in four European countries. Women who had normal ultrasound of the uterine cavity and history of two to four failed in vitro fertilisation cycles were randomised to have either outpatient hysteroscopy or no hysteroscopy in the month before starting the subsequent treatment cycle. The trial used allocation concealment and minimisation for key prognostic variables, including age, body mass index and basal follicle stimulating hormone level. The primary outcome was live birth rate. Secondary outcomes were pregnancy, implantation and miscarriage rates and hysteroscopy findings. The trial was registered on the ISRCTN Registry (#ISRCTN35859078).

Findings:

Seven hundred and two women younger than 38 years were randomly assigned between January 2010 and December 2013; 350 allocated to the outpatient hysteroscopy group and 352 to the control group. The live birth rate after in vitro fertilisation was 29% (102/350) in the hysteroscopy group and 29% (102/352) in the control group (relative risk [RR] 1·0; 95% confidence interval [CI] 0·79, 1·25, P=0·96). Hysteroscopy identified uterine abnormalities in 26% (85/323) of women. No hysteroscopy-related complications occurred. There were no significant differences in the pregnancy, implantation or miscarriage rates.

Interpretation:

Outpatient hysteroscopy before in vitro fertilisation treatment in women with normal ultrasound of the uterine cavity and a history of two to four failed in vitro fertilisation treatment cycles does not improve the live birth rate.

Funding:

The trial was funded by the European Society of Human Reproduction and Embryology, and the European Society for Gynaecological Endoscopy.

(word count = 300)

Introduction:

In vitro fertilisation treatment is utilised widely. In 2010, over 700,000 treatment cycles have been reported in the United States and Europe alone and the number of cycles is expanding steadily.[1,2] Despite technological advances, the live birth rate per cycle is modest and many patients remain infertile after multiple in vitro fertilisation attempts. Recurrent implantation failure, defined as two or more failed in vitro fertilisation embryo transfer cycles, [3] is distressing to patients and challenging to clinicians. [4] The aetiology of recurrent implantation failure could be attributed to either embryonic or uterine factors. Several interventions have been proposed to improve in vitro fertilisation outcome after multiple failed attempts, of which only few are evidence-based. [5,6]

Intra-uterine pathology has been reported in up to 25% of infertile women having in vitro fertilisation treatment and as high as 50% of women with recurrent implantation failure, leading to suggestion that correction of such pathology could improve treatment outcome.[7,8] Hysteroscopy allows visual assessment of the cervical canal and uterine cavity and provides the opportunity to perform corrective surgery in the same setting. [8-11] Routine outpatient hysteroscopy before starting in vitro fertilisation treatment has been proposed as a tool to confirm or restore normality of the uterine cavity and improve in vitro fertilisation treatment outcome. [12-14]

A systematic review of published studies has suggested that outpatient hysteroscopy performed in the menstrual cycle preceding an in vitro fertilisation treatment cycle could significantly increase the clinical pregnancy rate in women who had previously experienced recurrent implantation failure, even when no hysteroscopic abnormality was detected. [15] However, the review included five single-centre heterogeneous studies, of which only two were randomised trials lacking clear description of the method of randomisation, allocation concealment, adjustment for important confounding variables and sufficient live birth data, and thus suffering from a risk of bias. Therefore, conducting a robust multi-centre randomised study was needed to inform clinical practice. [13,15]

The aim of our study was to investigate whether outpatient hysteroscopy performed in the month before starting an in vitro fertilisation treatment cycle in women who had experienced two to four failed in vitro fertilisation treatment cycles could improve the treatment outcome. We conducted a multi-country multi-centre allocation concealed single-blind randomised controlled trial comparing the live birth rate after in vitro fertilisation with or without prior outpatient hysteroscopy (The TROPHY Trial). [16]

Methods

Study design:

The TROPHY study was a multi-centre allocation concealed single-blind randomised controlled trial conducted between January 2010 and December 2013 in eight European hospitals located in the United Kingdom (3 sites), Belgium (2 sites), Italy (2 sites) and the Czech Republic (1 site). The trial was registered on the ISRCTN Registry (#ISRCTN35859078). The study was approved by the United Kingdom Research Ethics Committee (reference: 09/H0804/32), and the ethics committees of participating hospitals.

Participants:

Women below the age of 38 years of age who had a normal transvaginal ultrasound appearance of the uterine cavity and previously had between two to four in vitro fertilisation treatment cycles ending in an embryo transfer but no pregnancy and who were undergoing a further treatment cycle of in vitro fertilisation were eligible to participate in the trial. Women aged 37 years were eligible to participate only if they had at least 8 oocytes retrieved in the previous in vitro fertilisation cycle. All participants gave written informed consent and were included in the study only once.

Women were excluded if they had less than two or more than four failed in vitro fertilisation cycles ending in an embryo transfer, a hysteroscopy within two months before randomisation, submucous or intramural uterine fibroids diagnosed by ultrasound to be distorting the uterine cavity, untreated tubal hydrosalpinges, or a body mass index above 35 kg/m^2.

Randomisation and masking:

Participants were randomised to receive either outpatient hysteroscopy or no hysteroscopy online via a secure internet facility in a 1:1 ratio through a third party independent Integrated Trial Management System (MedSciNet Clinical Trial Framework). A minimisation procedure using a computer-based algorithm was used to avoid chance imbalances, within the whole study participants and in each participating centre, in important prognostic variables including age (≤30 or 31-37 years), body mass index (<30 or 30-35 kg/m^2), number of previous failed in vitro fertilisation treatment cycles (2 or 3-4) and basal follicle stimulation hormone level (<10 or ≥10 iu/L). The randomised allocation was not given until eligibility and minimisation data had been completed. The trial was single-blind, where the embryologists involved in the embryo transfer procedures were blinded to patient's allocation in the trial. The physicians performing the embryo transfer procedure were not blinded to patient allocation and had the hysteroscopy findings accessible.

Procedures:

Women randomised to outpatient hysteroscopy had the procedure performed within 14 days of menstruation and started the in vitro fertilisation treatment cycle in the following month according to a standard in vitro fertilisation protocol.[17] Women randomised to the control group started the in vitro

fertilisation treatment cycle according to a standard in vitro fertilisation protocol without undergoing hysteroscopy.

Outpatient hysteroscopy:

Outpatient hysteroscopy was performed using a rigid 30° view 2.9 mm diameter hysteroscope with an atraumatic tip (TROPHYscope, Karl Storz, Tuttlingen, Germany) in a vaginoscopic approach. [14,16,18] The hysteroscope could be assembled with accessory sheaths in an active or passive position. Each hysteroscopy was started with the single-flow 2·9 mm instrument for inspection of the cervical canal and uterine cavity, and if necessary, the accessory diagnostic (3·7 mm) or operative (4·4 mm) sheath was moved forward to establish a double-flow mode and allow operative intervention using 5 French instruments (crocodile forceps, biopsy forceps and scissors). An isotonic solution (0.9% Normal saline or Ringer lactate) administered via a pressure-controlled pump or simple pressure cuff system was used to provide the lowest pressure required to distend the uterine cavity for adequate visualisation. No routine pre-operative analgesia, antibiotics, sedation or cervical preparation was used. A standardised protocol, data collection tool and clear description of possible abnormalities were provided to each participating centre.[16]After hysteroscopy, patients were observed in a recovery area before being allowed to leave the clinic.

In vitro fertilisation protocol:

The in vitro fertilisation treatment cycle was commenced in the menstrual cycle immediately following the outpatient hysteroscopy. The ovarian stimulation protocols used for the in vitro fertilisation treatment cycles were described previously.[17] Briefly, follicle stimulating hormone injections were started at a dose of 150-450 IU daily for multi-follicular ovarian stimulation. Final oocyte maturation was induced using 5,000-10,000 IU of human chorionic gonadotrophin when at least two 18 mm follicles were seen on ultrasound scanning. Ultrasound-guided oocyte retrieval was performed 34-38 hours following human chorionic gonadotrophin administration. Progesterone supplementation was used for luteal phase support and continued for up to eight weeks gestation if pregnancy had occurred. Embryo development and quality after fertilisation were assessed until transfer or freezing.[19] Between one and three embryos were transferred into the uterine cavity according to each participating centre's protocol.

Outcomes:

The primary outcome was the live birth rate (after 24 weeks gestation). Secondary outcomes were pregnancy (defined as positive human chorionic gonadotrophin test using commercial urinary testing kit), clinical pregnancy (defined as the observation of fetal cardiac activity on ultrasound scan four or more weeks after embryo transfer), implantation (defined as the presence of an intra-uterine gestational sac on ultrasound scan four or more weeks after embryo transfer) and miscarriage (defined as pregnancy loss before 24 weeks gestation) rates, abnormal hysteroscopy findings and

hysteroscopy-related complications. The implantation rate was calculated as the number of gestational sacs seen on ultrasound scanning divided by the number of embryos transferred.

Statistical Analysis:

Number of participants

It was calculated that to detect a minimally important difference of 10% increase in the live birth rate from 25% to 35%, for a double-sided alpha error of 5% and 80% power, it would be necessary to randomise 329 women each to the outpatient hysteroscopy and control groups (658 women in total). Assuming a drop-out rate of 5%, the number of participants required would be 694 in total.[20] The baseline live birth rate of 25% and the minimally important difference of 10% were based conservatively on the results of the studies included in the published systematic review[16] and following consultations with fertility practitioners. No replacement of trial subjects who had withdrawn from the study was planned as the analysis was performed according to intention-to-treat. Sample size calculations were carried out in Stata version 13·1 (Stata Corp, College Station, Texas, USA), following standard methods for two proportions without a continuity correction.

Baseline and outcome data were summarised separately. For normally distributed continuous variables, data were summarised as means with standard deviations. For non-normally distributed variables, data were reported as medians and inter-quartile ranges [IQR]. Categorical baseline and dichotomous data were reported as absolute numbers and percentages. The statistical procedures used for comparisons depended on the nature of the data; for dichotomous outcomes we used risk ratios calculated using binomial regression with a log link, and for continuous outcomes we used t-test if the observations in each trial arm were normally or near-normally distributed (or could be transformed to normality using a log transformation). If there was a suspicion of non-normality, boot-strapping with 500 replications was carried out as a sensitivity analysis.

Sub-group analysis

We gave emphasis to analysis within planned (a priori) sub-groups (namely normal versus abnormal hysteroscopic findings and analysis by receiving embryo transfer including receiving at least one top quality embryo[19]) with an interaction test. As sub-group analysis could suffer from false positive (due to multiplicity of comparisons) and false negative (due to reduced sample sizes) results, we determined the outcome of the trial in terms of the primary endpoint (live birth rate) and placed limited importance on sub-group analysis findings in relation to the overall findings. We hypothesised that outpatient hysteroscopy could be most beneficial in women who did not report a history of having hysteroscopy before randomisation, and used post-hoc sub-group analysis with an interaction test to assess the consistency of the intervention effect and for hypothesis generation only.

Study Oversight:

The TROPHY trial was conducted according to the Principles of Good Clinical Practice as defined in the Medicines for Human Use (Clinical Trials) Amended Regulations 2006, the Research Governance

Framework for Health & Social Care 2005 and the Data Protection Act. Trial oversight was provided by the Trial Steering Committee and an independent Data Safety Monitoring Committee. The Data Safety Monitoring Committee had independent members with clinical and statistical background, who had no conflict of interest relating to the two trial arms and no involvement in running any part of the trial. During the trial, the Data Safety Monitoring Committee reviewed unblinded outcome data for principal safety and efficacy end points.

Role of the funding source:

The European Society of Human Reproduction and Embryology and the European Society for Gynaecological Endoscopy provided funding for trial co-ordination and meetings. Karl Storz Company provided the hysteroscopy equipment for all centres and Tristel Solutions Limited (Snailwell, Cambridgeshire, United Kingdom) provided the equipment disinfecting systems for five of the eight participating centres. Neither the trial funders nor the medical products suppliers had any role in the study design, data collection, data analysis, data interpretation or writing of the report.

Results:

A total of 702 women were recruited to the TROPHY trial; 350 women to receive hysteroscopy and 352 women to receive no hysteroscopy before starting the in vitro fertilisation (with or without intracytoplasmic sperm injection) treatment cycle. The baseline characteristics of the study population were comparable across the two study groups (Table 1). The participants' flow in the study is shown in figure 1.

In the hysteroscopy group, 323/350 (93%) women received outpatient hysteroscopy, one had saline hysterosonography instead at her request, and 26 did not have a hysteroscopy (Figure 1). Of the 323 hysteroscopy procedures performed, no cavity access failure was encountered and one hysteroscopy was not completed due to insufficient visualisation. A cervical canal or uterine cavity abnormality was reported in 85 hysteroscopies (26%, Table 2). Of the 34 uterine cavity abnormalities detected, 15 were treated surgically including resection of eight endometrial polyps, four partial uterine septae, two submucosal fibroids and one T-shaped cavity. No surgical intervention was performed for dysmorphic (arcuate) uterine cavity (n=15), hemi-uterus (n=3) and one partial uterine septum. Four women in the control group had hysteroscopy at their request before starting the in vitro fertilisation treatment cycle. The median duration of the outpatient hysteroscopy procedure was seven minutes (IQR 5-10 minutes) and median duration to discharge after the procedure was 10 minutes (IQR 6-30 minutes). No hysteroscopy-related complications were reported.

Of the 702 randomised women, 640 (92%) started an in vitro fertilisation treatment cycle (Figure 1). The two study groups were similar in the in vitro fertilisation cycle characteristics (Table 3).

The live birth rate was 29% (102/350) in the hysteroscopy group and 29% (102/352) in the control group (RR 1·0, 95% CI 0·79, 1·25, P=0·96, table 4). The twin birth rate was 25% (25/102) in the hysteroscopy group and 28% (28/102, P=0·63) in the control group. There was no statistically significant heterogeneity in the live birth rate between the two groups amongst the eight hospitals participating in the trial (I^2 test of heterogeneity = 0%, P=0·62, figure 2). There was no statistically significant difference in the likelihood of a live birth between the two groups after adjusting for participants' age, body mass index, basal follicle stimulating hormone level, number of previous failed in vitro fertilisation treatment cycles and participating centre (adjusted RR 0·99, 95% CI 0·79, 1·24, P=0·95).

The pregnancy rate was 38% (133/350) in the hysteroscopy group and 39% (136/352) in the control group (RR 0·97, 95% CI 0·72, 1·32, P=0·86). The clinical pregnancy rate was 35% (121/350) in the hysteroscopy group and 33% (116/352) in the control group (RR 1·08, 95% CI 0·79, 1·47, P=0·65, table 4). The implantation rate was 29% in the hysteroscopy group and 30% in the control group in cycles reaching embryo transfer (RR 0.91, 95% CI 0·61, 1·37), and 32% in the hysteroscopy group and 32% in the control group in cycles where at least one top quality embryo was transferred (RR 1·0, 95% CI 0·65, 1·56). The overall pregnancy loss rate in the study was 25% (66/269), which included one ectopic pregnancy in the hysteroscopy group and two second-trimester pregnancy terminations due to severe fetal malformation (one in each group). The miscarriage rate was 22% (29/131) in the hysteroscopy group and 24% (33/135) in the control group (RR 0·91, 95% CI 0·59, 1·40, P=0·65).

Ten of the 301 women who received embryo transfer in the hysteroscopy group declined hysteroscopy (Figure 1). In the control group, four of the 290 women who received embryo transfer underwent hysteroscopy before starting the in vitro fertilisation cycle. Thus, a per-protocol analysis included 295 women in the hysteroscopy group and 296 women in the control group. According to that analysis, the live birth rate was 32% (94/295) in the hysteroscopy group and 33% (97/296) in the control group (RR 0·97, 95% CI 0·77, 1·23, P=0·81).

The live birth rate was similar in the subgroup of women who had a normal hysteroscopy (28%, 66/238) and in those who had an abnormal hysteroscopy (30%, 26/85), compared to the live birth rate in the control group (RR 0·96, 95% CI 0·74, 1·24, P=0·74, and RR 1·06, 95% CI 0·74, 1·51, P=0·76,

respectively). The live birth rate in the subgroup of women in whom a subtle endometrial abnormality was reported at hysteroscopy was 33% (14/41).

The live birth rate was similar in the subgroups of women who had two previous failed in vitro fertilisation treatment cycles in the hysteroscopy (28·4%, 52/183) and control (27%, 51/189) groups (RR 1·05, 95% CI 0·76, 1·46, P=0·75), and in those who had at least three previous failed in vitro fertilisation treatment cycles in the hysteroscopy (30%, 50/167) and control (31·3%, 51/163) groups (RR 0·96, 95% CI 0.69, 1·32, P=0·79).

Among women who did not report a history of undergoing a hysteroscopy over two months before randomisation, the live birth rate was 34% (65/193) in the hysteroscopy group and 31% (61/198) in the control group (RR 1·09, 95% CI 0·82, 1·46, interaction test P=0.48).

Discussion:

This large multi-centre allocation concealed randomised trial showed that routine outpatient hysteroscopy performed before starting an in vitro fertilisation treatment cycle in women who had normal ultrasound scan of the uterine cavity and a history of recurrent implantation failure does not improve treatment outcome. The study results do not support earlier suggestions that outpatient hysteroscopy could improve the success rate of in vitro fertilisation treatment, even when hysteroscopy reveals normal findings. We found no difference in the live birth rate after in vitro fertilisation treatment in women who had a normal hysteroscopy compared with women who had no hysteroscopy.

Previous studies have hypothesised that the beneficial effect of hysteroscopy on in vitro fertilisation outcome could be mediated through the treatment of unsuspected uterine pathology identified at hysteroscopy.[21] Our study identified cervical or uterine cavity abnormalities in 26% of the hysteroscopies performed. However, in two-thirds (57/85) of these hysteroscopies, the reported abnormalities were not treated, because they were considered either untreatable, such as deviated or shortened cervical canal and hemi-uterus, or of undetermined clinical significance, such as dysmorphic (arcuate) uterine cavity and subtle endometrial abnormality.[22] Given the small number of uterine cavity abnormalities treated in the hysteroscopy group, the role of hysteroscopic correction of specific uterine cavity abnormalities in improving in vitro fertilisation outcome could not be determined based on the study results. Therefore, future research in which assessment of the uterine cavity is needed could include 3D ultrasound scanning in order to address the effectiveness of surgical correction of specific uterine cavity abnormalities before in vitro fertilisation treatment, and develop

universally-agreed guidelines to improve inter-observer agreement on the diagnosis of such abnormalities to further refine clinical practice. [23-25]

Hysteroscopy has been proposed to improve in vitro fertilisation outcome by stimulation of the endometrium through surface injury. This has been suggested to increase the likelihood of embryo implantation in the subsequent in vitro fertilisation treatment cycle.[26,27] Endometrial injury is not a routine step of outpatient hysteroscopy and could be performed without hysteroscopic guidance. It is plausible that the smaller diameter of the hysterocope used in our study (2·9mm), compared to the diameter of the hysterscopes used in previous studies (5mm), had caused less endometrial surface injury, resulting in a milder degree of endometrial stimulation for implantation. Nevertheless, this may not be a significant factor in light of recent evidence,[28,29] although further research is still required in this area.

It has also been postulated that since a difficult embryo transfer procedure could compromise in vitro fertilisation outcome, performing a hysteroscopy before starting a treatment cycle could facilitate future embryo transfer, via amelioration of cervical canal obstruction and optimisation of embryo transfer procedure, and thus improve treatment outcome.[15] Our study was the first randomised trial to record the clinician's assessment of the ease of the embryo transfer procedure in women with recurrent implantation failure, and found similar percentage of easy embryo transfer procedures in the two study groups (93% vs 94%).

In this study, 44% of women had a history of previously undergoing a hysteroscopy over two months before recruitment into the trial, which could account for the low prevalence of treatable uterine cavity abnormalities in the hysteroscopy group. It could be argued that those who did not have a history of previous hysteroscopy could benefit most from the procedure when performed just before starting the in vitro fertilisation treatment cycle. However, there was no significant difference in the live birth rate between the two study groups amongst those who did not have a history of previous hysteroscopy prior to recruitment into the trial, thus re-enforcing the main study results. This observation warrants further investigation in future studies.

The TROPHY trial has several strengths. In addition to the large number of women recruited into the trial, it addressed the substantive methodological limitations of previously published studies, via its multicentre design, robust randomisation and allocation concealment to eliminate selection bias,[30] and minimisation for key prognostic factors to achieve balanced treatment allocation at baseline. Important confounding variables such as duration of infertility, smoking habits, presence of uterine

fibroids, pre-treatment antral follicle count and in vitro fertilisation treatment cycle characteristics, were similarly distributed between the two study groups. Blinding of the embryologists involved in the embryo transfer procedure to the assignment of the treatment group was employed to reduce performance bias. Furthermore, the multi-country design of the study ensured that the results are applicable to different in vitro fertilisation settings, thus enhancing the generalisability and validity of the study conclusions.

In summary, it is important that routine interventions before in vitro fertilisation treatment are supported by robust evidence of effectiveness. The TROPHY study demonstrates that outpatient hysteroscopy in the month before starting an in vitro fertilisation treatment cycle in women younger than 38 years with normal ultrasound scan of the uterine cavity and history of two to four failed in vitro fertilisation treatment cycles does not increase the live birth rate.

(word count = 3486)

Acknowledgements:

We are grateful this study was supported by the European Society of Human Reproduction and Embryology (ESHRE), and the European Society for Gynaecological Endoscopy (ESGE). We thank all of the women who participated in this study and the following investigators for supervising recruitment and randomisation at study centres: Dr Jyotsna Pundir, Ms Jean Bvumbe, Dr Madhurima Rajkhowa, Dr Spyridon Chouliaras, Dr Yves Van Belle at The European Academy of Gynaecological Surgery, and Ms Rhona O'Flaherty at the ESGE Central Office. We also thank all TROPHY research nurses who assisted data collection. We are grateful to Emeritus Professor Peter Braude, and Mrs Annette Briley for chairing the data monitoring committee, to Professor Justin Clark for advice and assistance to design the study, and to Helen Williams at the University of Birmingham, for assistance with the final preparation of the manuscript. Finally, we thank all those not otherwise mentioned above who have contributed to the TROPHY study.

Author contribution:

Tarek El-Toukhy, Rudi Campo, Yacoub Khalaf and Arri Coomarasamy:
Conceived and designed the study, designed the statistical analysis of primary and secondary trial outcomes along with the trial statistician, co-ordinated the practical conduct of the study including recruitment and data collection, contributed to the Trial Steering Committee, co-ordinated the analyses, and produced and approved the final report.

Carla Tabanelli, Luca Gianaroli, Sylvie Gordts, Stephan Gordts, Greet Mestdagh, Tonko Mardesic, Jan Voboril, Gian Luigi Marchino, Chiara Benedetto, Talha Al-Shawaf, Luca Sabatini and Hoda Harb:

Contributed to the design of the study, co-ordinated the practical conduct of the study including conducting recruitment and data collection, contributed to the analyses and commented on and approved the final report.

Marco Gergolet and Grigoris Grimbizis:

Contributed to the design of the study, provided advice and oversight to the conduct of the study, contributed to the analyses and commented on and approved the final report.

Paul T Seed:

Designed the statistical analysis of primary and secondary trial outcomes, produced and co-ordinated the analyses, and commented on and approved the final report.

Declaration of Interest:

Dr R Campo has a patent for Trophy hysteroscope with royalties paid and received educational grant to the research program of the European Academy of Gynaecological Surgery by Karl Storz Endoscopy, MSD and Tristel.

References:

1) Sunderam S, Kissin DM, Crawford S, Anderson JE, Folger SG, Jamieson DJ, Barfield WD; Division of Reproductive Health, National Center for Chronic Disease Prevention and Health Promotion, CDC. Assisted reproductive technology surveillance -- United States, 2010. *MMWR Surveill Summ* 2013; **62** (9): 1–24.

2) Kupka MS, Ferraretti AP, de Mouzon J, Erb K, D'Hooghe T, Castilla JA, Calhaz-Jorge C, De Geyter C, Goossens V; European IVF-Monitoring Consortium, for the European Society of Human Reproduction and Embryology. Assisted reproductive technology in Europe, 2010: results generated from European registers by ESHRE. *Hum Reprod* 2014: **29** (10): 2099–113.

3) Polanski LT, Baumgarten MN, Quenby S, Brosens J, Campbell BK, Raine-Fenning NJ. What exactly do we mean by 'recurrent implantation failure'? A systematic review and opinion. *Reprod Biomed Online* 2014; **28** (4): 409–23.

4) Coughlan C, Walters S, Ledger W, Li TC. A comparison of psychological stress among women with and without reproductive failure. *Int J Gynaecol Obstet* 2014; **124** (2): 143–7.

5) Zeyneloglu HB, Onalan G. Remedies for recurrent implantation failure. *Semin Reprod Med* 2014; **32** (4): 297–305.

6) Papathanasiou A, Bhattacharya S. Prognostic factors for IVF success: diagnostic testing and evidence-based interventions. *Semin Reprod Med* 2015; **33** (2): 65–76.

7) Moini A, Kiani K, Ghaffari F, Hosseini F. Hysteroscopic findings in patients with a history of two implantation failures following in vitro fertilization. *Int J Fertil Steril* 2012; **6** (1): 27–30.

8) Cenksoy P, Ficicioglu C, Yıldırım G, Yesiladali M. Hysteroscopic findings in women with recurrent IVF failures and the effect of correction of hysteroscopic findings on subsequent pregnancy rates. *Arch Gynecol Obstet* 2013; **287** (2): 357–60.

9) Dicker D, Ashkenazi J, Feldberg D, Farhi J, Shalev J, Ben Rafael Z. The value of repeat hysteroscopic evaluation in patients with failed in vitro fertilisation cycles. *Fertil Steril* 1992; **58**: 833–5.

10) La Sala GB, Montanari R, Dessanti L, Cigarini C. The role of diagnostic hysteroscopy and endometrial biopsy in assisted reproductive technologies. *Fertil Steril* 1998; **70**: 378–80.

11) Levi-Setti PE, Colombo GV, Savasi V, Bulleti C, Albani E, Ferrazzi E. Implantation failure in assisted reproduction technology and a critical approach to treatment. *Annals NY Acad Sci* 2004; **1034**: 184–99.

12) Golan A, Ron El R, Herman A, Soffer Y, Bukovsky I, Caspi E. Diagnostic hysteroscopy: Its value in an in- vitro fertilisation/ embryo transfer unit. *Hum Reprod* 1992; **7**: 1433–4.

13) Carneiro MM. What is the role of hysteroscopic surgery in the management of female infertility? A review of the literature. *Surg Res Pract* 2014; **2014**: 105412.

14) Campo R, Meier R, Dhont N, Mestdagh G, Ombelet W. Implementation of hysteroscopy in an infertility clinic: The one-stop uterine diagnosis and treatment. *Facts Views Vis Obgyn* 2014; **6** (4): 235–9

15) El-Toukhy T, Sunkara SK, Coomarasamy A, Grace J, Khalaf Y: Out- patient hysteroscopy and subsequent IVF cycle outcome: a systematic review and meta-analysis. *Reprod BioMed Online* 2008, **16** (5): 712–9.

16) El-Toukhy T, Campo R, Sunkara SK, Khalaf Y, Coomarasamy A. A multi-centre randomised controlled study of pre-IVF outpatient hysteroscopy in women with recurrent IVF implantation failure: Trial of Outpatient Hysteroscopy - [TROPHY] in IVF. *Reprod Health* 2009; **6**: 20.

17) Sunkara SK, Coomarasamy A, Faris R, Braude P, Khalaf Y. Long gonadotropin-releasing hormone agonist versus short agonist versus antagonist regimens in poor responders undergoing in vitro fertilization: a randomized controlled trial. *Fertil Steril* 2014; **101** (1): 147–53.

18) Campo R, Van Belle Y, Rombauts L, Brosens I, Gordts S. Office mini-hysteroscopy. *Hum Reprod Update* 1999; **5**: 73–81.

19) Cutting R, Morroll D, Roberts SA, Pickering S, Rutherford A; BFS and ACE. Elective single embryo transfer: guidelines for practice British Fertility Society and Association of Clinical Embryologists. *Hum Fertil (Camb)* 2008; **11** (3): 131–46.

20) Agresti A. Categorical Data Analysis. 3rd edition. Hoboken, NJ: Wiley, 2013.

21) Fatemi HM, Kasius JC, Timmermans A, van Disseldorp J, Fauser BC, Devroey P, Broekmans FJ. Prevalence of unsuspected uterine cavity abnormalities diagnosed by office hysteroscopy prior to in vitro fertilisation. *Hum Reprod* 2010; **25** (8): 1959–65.

22) Galliano D, Bellver J, Díaz-García C, Simón C, Pellicer A. ART and uterine pathology: how relevant is the maternal side for implantation? *Hum Reprod Update* 2015; **21** (1): 13–38.

23) Bosteels J, Kasius J, Weyers S, Broekmans FJ, Mol BW, D'Hooghe TM. Hysteroscopy for treating subfertility associated with suspected major uterine cavity abnormalities. *Cochrane Database Syst Rev* 2015 Feb 21; **2**: CD009461.

24) Kasius JC, Broekmans FJ, Veersema S, Eijkemans MJ, van Santbrink EJ, Devroey P, Fauser BC, Fatemi HM. Observer agreement in the evaluation of the uterine cavity by hysteroscopy prior to in vitro fertilization. *Hum Reprod* 2011; **26** (4): 801–7.

25) Grimbizis FG, Gordts S, Di Spiezio Sardo A, Brucker S, De Angelis C, Gergolet M, Li TC, Tanos V, Brölmann H, Gianaroli L, Campo R. The ESHRE-ESGE consensus on the classification of female genital tract congenital anomalies. *Gynecol Surg* 2013; **10**: 199–212.

26) Potdar N, Gelbaya T, Nardo LG. Endometrial injury to overcome recurrent embryo implantation failure: a systematic review and meta-analysis. *Reprod Biomed Online* 2012; **25**(6): 561–71.

27) Nastri CO, Lensen SF, Gibreel A, Raine-Fenning N, Ferriani RA, Bhattacharya S, Martins WP. Endometrial injury in women undergoing assisted reproductive techniques. *Cochrane Database Syst Revi* 2015, **Issue 3**. Art. No.: CD009517.

28) Yeung TW, Chai J, Li RH, Lee VC, Ho PC, Ng EH. The effect of endometrial injury on ongoing pregnancy rate in unselected subfertile women undergoing in vitro fertilization: a randomized controlled trial. *Hum Reprod* 2014; **29** (11): 2474–81.

29) Panagiotopoulou N, Karavolos S, Choudhary M. Endometrial injury prior to assisted reproductive techniques for recurrent implantation failure: a systematic literature review. *Eur J Obstet Gynecol Reprod Biol* 2015; **193**: 27–33.

30) Lachin JM. Statistical properties of randomization in clinical trials. *Control Clin Trials* 1988; **9** (4): 289–311.

Table 1. Baseline patient demographic characteristics. Results are given as % (n/N) unless otherwise stated.

Characteristic	Hysteroscopy group	Control group
Mean (±SD*) female age at randomisation (range 23-37 years)	32·7 ± 3·1	32·7 ± 3·1
Female age ≥30 years	82·6 % (289/350)	83·8 % (295/352)
<30 years	17·4 %	16·2 %
30-35 years	58·3 %	62·2 %
>35 years	24·3 %	21·6 %
Mean (±SD) female body mass index at randomisation (range 17-35)	23·4 ± 3·5	23·3 ± 3·4
Female body mass index 30-35 kg/m^2	4·6 % (16/350)	4·8 % (17/352)
Female smoking status	4·6 % (16/350)	3·7 % (13/352)
Mean (±SD) duration of infertility, years	4·2 ± 3·4	4·2 ± 2·8
Cause of Infertility		
Male factor	45 % (157/350)	45 % (159/352)
Tubal Factor	17 % (61/350)	15 % (53/352)
Anovulation	6% (21/350)	7 % (26/352)
Endometriosis	11 % (39/350)	7 % (26/352)
Combined	6 % (20/350)	7 % (26/352)
Unexplained	15% (52/350)	18 % (62/352)
Previous pregnancy	35% (123/350)	35% (122/352)
Previous live birth	10 % (34/350)	11 % (37/352)
Mean (±SD) number of previous failed IVF cycles	2·7 ± 0·9	2·7 ± 1·0
Mean (±SD) failed fresh IVF cycles	1·9 ± 0·8	1·9 ± 0·7
Mean (±SD) failed frozen IVF cycles	0·8 ± 0·8	0·9 ± 0·9
Presence of uterine fibroids	4% (15/350)	4 % (14/352)
intramural	6/15	7/14
subserosal	7/15	6/14
intramural and subserosal	2/15	1/14
Mean largest diameter of fibroid (SD) mm	22·3 (13·4)	23·7 (18·1)
Mean basal follicle stimulating hormone level iu/L (range 1-16)	6·1 ± 2·4	6·3 ± 2·5
Basal follicle stimulating hormone level ≥10 iu/L	5 % (17/350)	5 % (18/352)
Mean antral Follicle count[§]	15 ± 7	16 ± 8
Previous hysteroscopy	45 % (157/350)	44 % (154/352)
Previous uterine surgery	6·3 % (22/350)	6·5 % (23/352)
Myomectomy	6	4
Caesarean section	2	2
Correction of congenital anomaly	2	3
Removal of conception products	1	2
Removal of polyp(s) or scarring	4	6
Cervical dilation, cauterisation or excision of transformation zone	7	6

* SD denotes standard deviation

[§] Estimates checked by boot-strapping and no important difference found.

Table 2. Abnormal hysteroscopic findings in the hysteroscopy group.

Hysteroscopic finding	**Number**
Cervical abnormalities (4%, 14/323)	
Stenosis of external os	3
Stenosis of internal os	4
Cervical canal adhesions	3
Cervical canal deviation (retroversion)	2
Polyp	1
Shortened cervical canal	1
Uterine cavity abnormality (10.5%, 34/323)	
Dysmorphic (arcuate) cavity	15
Hemi-uterus	3
Endometrial polyp(s)	8
Partial uterine septum	5
Submucous fibroid	2
T-shaped uterine cavity	1
Subtle endometrial abnormality (11.5%, 37/323)	
Hypervascularisation	20*
Mucosal elevation	12
Micro-polyps	3
Pale endometrium	3
Endometrial defect	2
Single adhesion band	1

* four patients also had dysmorphic (arcuate) uterus

Table 3. In vitro fertilisation cycle and embryologic characteristics. Results are given as % (n/N) unless otherwise stated.

IVF cycle feature	Hysteroscopy	Control group	Mean difference (95% CI*)	P-value
IVF treatment protocol Long GnRH agonist Short GnRH agonist Short GnRH antagonist Other	 48 % (156/322) 14 % (45/322) 35 % (112/322) 3 % (9/322)	 50 % (158/318) 16 % (50/318) 30 % (97/318) 4 % (13/318)		
Percentage of cycles using recombinant gonadotrophins	62%	67%		0·21
Mean (±SD) total dose of gonadotrophins used	2425 ± 1335	2281 ± 1269	144 (-57, 344)	0·16
Mean (±SD) number of oocytes retrieved	10·5 ± 5·6	10·5 ± 6·1	-0·06 (-1·0, 0·89)	0·91
Percentage of cycles using ICSI for oocyte fertilisation	78%	78%		0·84
Mean (±SD) number of oocytes fertilised normally	6·1 ± 4·0	5·8 ± 4·1	0·23 (-0·42, 0·88)	0·49
Number of IVF cycles reaching embryo transfer	301	290		
Mean number of embryos transferred 1 embryo transferred 2 embryos transferred 3 embryos transferred	1·8 ± 0·5 26% (78/301) 70% (212/301) 4% (11/301)	1·8 ± 0·5 26% (75/290) 70% (203/290) 4% (12/290)	0·02 (-0·10, 0·06)	0·66
Day of embryo transfer Day 2 Day 3[§] Day 5/6 Not known	 24 % (73/301) 35 % (104/301) 40 % (120/301) 1 % (4/301)	 24 % (70/290) 35 % (99/290) 40 % (118/290) 1 % (3/290)		
Easy embryo transfer[α]	93% (280/301)	94% (271/290)	-0·4 (-2·4, 8·9)	0·83
Embryo transfers with at least one top quality embryo[¶]	77% (232/301)	79% (236/290)	-2% (-8, 5)	0·58
Cycles with surplus embryos frozen	45% (137/301)	41% (118/290)	-4% (-3·4, 12·4)	0·26

* CI denotes confidence interval

[§] Including embryo transfer on day 4

[α] Defined as a straightforward transfer without encountering any difficulty or requiring the use of rigid stylet or application of a volsellum

[¶] An embryo was considered of top quality if it had four cells on day 2 or seven to eight cells on day 3, with even cell size and no or less than 10% cytoplasmic fragmentation by volume, or if it had reached the expanded blastocyst stage on day 5 or 6 after fertilization with prominent and compact inner cell mass and many identical trophectoderm cells forming a continuous layer[19]

Table 4. Rates of pregnancy and live birth in the two study groups. Results are given as % (no.).

Variable	**Hysteroscopy group**	**Control group**	**Relative risk (95% CI*)**	**P-value**
Rate / patient randomly assigned to intervention	N=350 **% (no.)**	N=352 **% (no.)**		
Pregnancy	38 (133)	39 (136)	0·97 (0·72, 1·32)	0·86
Clinical pregnancy	35 (121)	33 (116)	1·08 (0·79, 1·47)	0·65
Live birth	29 (102)	29 (102)	1·0 (0·79, 1·25)	0·96
Rate / patient receiving embryo transfer	N=301 **% (no.)**	N=290 **% (no.)**		
Pregnancy	42 (125)	44 (128)	0·94 (0·78, 1·13)	0·52
Clinical pregnancy	38 (114)	38 (110)	0·99 (0·81, 1·22)	0·99
Live birth	32 (95)	33 (96)	0·95 (0·76, 1·20)	0·69
Rate / patient receiving at least one top quality embryo	N=232 **% (no.)**	N=236 **% (no.)**		
Pregnancy	45 (104)	46 (109)	0·97 (0·80, 1·18)	0·77
Clinical pregnancy	42 (97)	42 (98)	1·01 (0·84, 1·21)	0·99
Live birth	35 (82)	36 (86)	0·98 (0·81, 1·24)	0·81

* CI denotes confidence interval